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Humoral and Mucosal-Humoral Immune Response to a *Salmonella* vaccination Program in Broiler Breeders

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Abstract: Although vaccination against *Salmonella* has been used more frequently in broiler breeders in recent years, there is limited information in the literature demonstrating the immunological response of combinations of live and killed whole cell vaccines. The present research assesses the immunological response generated by three different vaccination protocols. Treatment vaccines consisted of a live Aro-A mutant commercial *Salmonella* Typhimurium (ST) vaccine (Fort Dodge Animal Health) and a commercially prepared killed vaccine consisting of a pool of *Salmonella* serovars Berta (D_1), Heidelberg (B) and Kentucky (C_2). Three vaccination treatments using live, killed or a live-killed combination plus a non-vaccinated control were evaluated. Serum (SER), Crop Lavage (CL), Gut Lavage (GL), hatchling serum and egg yolk were tested for specific IgA and IgG anti-*Salmonella* Enteritidis (SE) or *Salmonella* Typhimurium lipopolysaccharide (SELPS or STLPS, respectively) antigen by indirect ELISA. Immunological response was stronger on STLPS than SELPS. IgA of SER and CL were short-lived peaks after the first killed vaccine, with Optical Densities (OD) greater than 1.000. A short-lived peak of IgG of CL on STLPS (OD>1.500) was also observed. Strong GL IgG after first live and both killed vaccine events were observed (OD>1.000), with the response to the killed preparation enduring longer. SER IgG responses observed after killed vaccination lasted throughout 40 wks of age with no demonstrable differences between treatments. Hatchling serum and egg yolk IgA were negligible and IgG was comparable among all treatments throughout time. Results confirm that killed antigen is vital in eliciting adequate IgG in serum and gut. Live vaccination with Aro-A mutant ST vaccine enhances gut IgG and possibly aids in conferring adequate immunity during the breeder's first wks of life.

Key words: *Salmonella*, mucosal-humoral, mucosal, immune response, vaccine and broiler-breeders

Introduction

Mandatory implementation of Hazard Analysis and Critical Control Points (HACCP) as the primary tool for pathogen reduction in the processing plant has increased pressure on poultry processors to minimize any potential source of *Salmonella* coming into the plant (USDA, 1996). Risk analysis for the processing plant has shown water, environment, live haul transportation and fomites in general, as well as carrier birds, to be the main sources of *Salmonella* contamination. Of these factors, live transport equipment and carrier birds are possibly the major culprits (McCapes *et al*., 1998).

Salmonella vaccination studies resulted in the development of live vaccines as well as killed bacterins, which are both commonly used in the field for layer, breeder and commercial broilers. The bacterin type used in commercial layer operations is generally restricted to SE bacterins, since egg transmission of this potential human pathogen is the primary concern in layer flocks. In contrast, the most widely used bacterins in broiler breeder settings are traditional water-in-oil autogenous emulsions, generally manufactured by a commercial vaccine manufacturer for a particular customer and using a blend of two or three of the most prevalent serovars commonly encountered in the field by the customer. The goal of vaccination in broiler breeder operations is to curb the incidence of vertical transmission of field *Salmonella* to the progeny. Reduction of vertical transmission may have some effect on overall broiler performance depending on the serovar's degree of virulence and host adaptation, but more importantly, may help reduce the incidence of *Salmonella* carried into the processing plant.

Gene deletion ()) used as a tool for attenuation of vaccine strain candidates has seen diverse approaches. A licensed ST live vaccine for poultry was developed by deletion of the aro-A gene, which encodes 5-enolpyruvylshikimate-3-phosphate synthase, an enzyme involved in synthesis of the aromatic amino acid precursor chorismate (Hosieth and Stocker, 1981; Dougan *et al*., 1987; Dougan *et al*., 1988). Other gene deletion mutants (Aro-C and Aro-D, encoding for chorismate synthase and 3-dehydroquinase) involved in chorismate synthesis and double and triple-deletion combinations have been developed in ST and *Salmonella* serovar typhi, the causative agent of human typhoid (Chatfield *et al*., 1992; Hone *et al*., 1991). Double deletion of genes coding for receptor protein of cAMP

and adenylate cyclase ( )crp and)cya) yielded a severely attenuated
ST. Deletion of these genes affects carbohydrate metabolism, affecting expression of fimbriae and flagella (Curtiss *et al*., 1988).
Although cell-mediated immunity is widely recognized as an important mechanism in the bird's response to *Salmonella* challenge (Arnold and Holt, 1995), specific aspects of this response are still largely unknown (Zhang-Barber *et al*., 1999; Lillehoj and Okamura, 2003) and no practical test for cell-mediated immunity in the field exists. Measurement of antibody as an indicator of humoral immune response by ELISA is still the most widely used tool to monitor a flock's immune status. Cell-mediated responses may better reflect an animal's potential resistance to challenge compared to humoral response (Lee *et al*., 1983). However, a genetic link to antibody production correlation, as well as, greater antibody production to decreased *Salmonella* colonization correlation have been demonstrated (Kaiser and Lamont, 2001; Kaiser *et al*., 2002), showing that antibody monitoring is a practical and valuable tool for relating antibody response to resistance to challenge. Commercially-available kits and research-type protocols exist for measuring anti-*Salmonella* immunoglobulin in serum, with commercial ELISA assays measuring IgG on flagellin-coated plates and research ELISA assays capable of measuring IgA or IgG on LPS or flagellin-coated plates (Holt and Porter, 1993; Idexx, 2004).
Few long-term studies focusing on live *Salmonella* vaccination and effects on the chicken's immune response have been conducted (Hassan and Curtiss, 1997) and to our knowledge, no reports using protocols combining live and killed vaccines with commercial breeds under industry-type vaccine delivery and rearing conditions exist. The few long-term studies have used)cya)crp mutants using direct oral gavaging of the vaccine and assessed protection to homologous serovar Typhimurium and heterologous serovar Enteritidis (Hassan and Curtiss, 1997). Although a degree of cross-protection of live vaccines on subsequent challenge with heterologous serotypes has been demonstrated (Hassan and Curtiss, 1994; Hassan and Curtiss, 1997), efficacy of protection is affected by the particular vaccine and challenge strains (Zhang-Barber *et al*., 1999). Efficacy of protection would be expected to decrease as antigenic differences between vaccine and challenge strains increase.
The gut-associated lymphoid tissues are the secondary lymphoid tissues located in the alimentary tract and intestinal mucosa and classically associated with intestinal Peyer's patches and cecal tonsils (Schat and Myers, 1991). More recent studies have focused attention on the crop as a possible site for mucosal immunity. A procedure for harvesting immunoglobulins from chicken's crops was developed (Holt *et al*., 2002) and production of crop anti-SE IgA following infection has been demonstrated (Seo *et al*., 2002; Seo *et al*., 2003a). The crop-lavage technique provides a useful tool in studying humoral mucosal responses at the alimentary tract level and similar lavage procedures may be used in obtaining samples for intestinal antibody monitoring. In this case however, euthanization of the chicken to be sampled is necessary prior to the intestinal lavage procedure. Studying differences in serum and humoral mucosal antibody dynamics may provide further insight to the bird's response to *Salmonella* vaccination and challenge.
Primary airborne exposure in hatching cabinets (Cason *et al*., 1994) or in the houses can precede intestinal *Salmonella* colonization of previously uninfected chickens. Although *Salmonella* exposure in commercial broiler and breeder flocks requires colonization of the intestinal tract, environment reduction of S*almonella* by use of an electrostatically charged apparatus resulted in decreased incidence of infection, demonstrating the importance of airborne *Salmonella* transmission in broiler breeder houses (Richardson *et al*., 2003a; Richardson *et al*., 2003b). Commercially-available live *Salmonella* vaccines are massively aerosolized at the hatchery or on arrival to the farm and sometimes a second application is given by aerosol or drinking water. Our studies therefore, focused on profiling humoral and gut mucosal IgG and IgA responses of broiler breeders subjected to 3 different vaccination protocols under vaccination and rearing conditions closely resembling today's industry practices.

Materials and Methods

Chickens and premises: One thousand female and one hundred and fifty male day-old Cobb x Cobb broiler breeder parents were obtained from a major commercial broiler breeder supplier and placed at the University of Georgia's Poultry Science Research facilities. Females came from a 57 wk-old and males from a 34 wk-old grandparent stock, respectively. After randomization, chicks were placed in four separate units consisting of identical environmentally-controlled rooms each having independent mechanical trough feeding systems and nipple drinkers. Rooms were negatively ventilated; force air heated or evaporatively cooled; and these systems were electronically controlled. Air inlets and exhausts were fitted with light traps. Light was provided by high pressure sodium and fluorescent bulbs. Each room was 9.1m wide x 7.3m deep and 3.05m high. All rooms and equipment were washed and foam-disinfected with BioSentry 904® (DuPont Animal Health, Inc., Sudbury, Suffolk, UK) according to the manufacturer's specifications. Approximately 3 inches of fresh pine shavings were placed on the previously cleaned premises and formalin allowed to react with potassium dichromate at an approximate concentration of 10g of formalin per cubic meter of the premise. Drag swabs of equipment and premises 4 d after sanitation

Table 1: Vaccination treatments at different breeder ages

	Breeder Age (d)			
Treatments	1	21	77	119
C				
2L2K	L	L	K	K
3L1K	L	L	L	K
2K			K	K

C = non-vaccinated controls. 2K = Killed vaccines given on wk 11 and 17; 2L2K = live vaccines given on d 1 and 21 and killed vaccines given on wk 11 and 17; 3L1K = live vaccines on d 1, 21 and wk 11 and 1 killed vaccine given on wk 17. L = live vaccine; K = killed vaccine

Table 2: Age of chickens and samples taken for antibody assays

	Sample				
Breeder Age (d)	Breeder Serum	Crop Lavage	Gut Lavage	Egg Yolk	Serum Hatchling
1	S	S	S		
21	S	S	S		
42	S	S			
77	S	S	S		
98	S	S			
119	S	S	S		
154	S	S	S		
189	S	S	S	S	S
238	S	S	S	S	S
280	S	S	S	S	S

S = Sampled

were cultured for *Salmonella* spp., by direct plating on Brilliant green-sulfa agar (BGS, Becton-Dickinson, Franklin Lakes, NJ), or pre-enriched followed by enrichment in tetrathionate broth, Hajna formulation (TT, Becton-Dickinson, Franklin Lakes, NJ) before plating, yielding negative results. On arrival of chicks to the farm, chick box liners were cultured for *Salmonella* and $1m^2$ live paper liners placed wkly under feeder troughs and cultured for *Salmonella* monitoring on wk 1, 3, 6, 11, 14 and 17 of age.

Vaccines: On arrival to the farm, female chicks were randomized into four treatments, consisting of a non-vaccinated control, a two-live/two-killed (2L2K), a three-live/one-killed (3L1K) and a two-killed (2K) group. Live vaccine was Poulvac-ST ® (Fort Dodge Animal Health Inc, Overland Park, KS), an Aro-A serovar Typhimurium mutant. The live vaccine was given as coarse spray while inside chick boxes at day-of-age, or via drinking water at wk 3 or 11 of age. Killed vaccine was a water-in-oil emulsion of a blend of serovars Heidelberg (group B), Kentucky (group C2) and Berta (group D1), an antogenous commercially preparation (Lohmann Animal Health International, Gainesville, GA) for a major broiler grower in the southeast. Killed vaccines were given subcutaneously on wks 11 or 17 of age. Vaccination treatments and days of delivery are shown in Table 1. Males were raised in a separate identical unit. Pullets were fed *ad libitum* for the first four wks and entered a skip-a-day feed restriction program until moved to the production units. Amounts of feed delivered were calculated weekly based on weekly body weights. Lighting was 24 hr for the first day and was reduced to 8 h at 4 wks, followed by light stimulation once pullets were 21 wk of age. Feeding and lighting programs closely resembled current broiler breeder husbandry practices.

At 18 wks of age, pullets were moved to almost identical rooms equipped with nests on laterally placed slats on 2/3 of the total floor area and a central non-slatted mating/scratch area. Mechanical feeding chain troughs, automatic nipples and belt-conveyed nests resembled a typical broiler breeder house. Males were introduced a few days after the females.

Humoral and mucosal samples: Blood, crop lavage and gut lavage samples (n = 10/sample type/day) were collected periodically to profile immunoglobulin concentrations on each sample type through time. Blood samples were obtained from the brachial vein of chickens, except for the day-of-age samples which were obtained from the jugular vein. Crop lavage samples were taken according to Holt *et al*. (2002). Briefly, lavage solution consisting of a 1M Tris/glycine buffer (Sigma Chemical Co., St Louis, MO) with 0.25% Tween20 (Sigma Chemical Co) was flushed into the crop and then gently massaged and the solution aspirated back into the syringe. Five ml of lavage solution was administered using 3/16 inch outer diameter Tygon™ tubing when sampling birds 6 wks or older, but only 2.5-5ml of lavage solution using a 1/8 inch tubing was used for younger birds. Gut lavage samples were obtained after euthanizing a subset of chicks. The small intestine was carefully excised at the ventriculo-duodenal and at the ileo-cecal junctions. The section was removed and flushed with 10ml of lavage solution by inserting a feeding needle (Propper, New Hyde Park, New York) through the ileal extreme and collecting flushed material through the duodenal extreme into 15ml centrifuge tubes. Samples were kept on ice until reaching the laboratory, where they were immediately centrifuged at 2,500g for 10 min. The supernatant was frozen at-7°C until the ELISA assay procedure. Once in production, egg yolk and hatchling serum samples were taken. Immunoglobulin from egg yolk was extracted using the oily-acid protocol of Seo *et al*., 2003b. Table 2 summarizes samples taken at each bird age.

ELISA assays: Indirect ELISA assays were conducted using a method similar to that of Holt *et al*., 1993. However, in the original assay, antibody capture using *S.* Typhimurium (ST) flagellar antigens were described while the current research used *S.* Enteritidis (SE) or ST LPS as capture antigens. Plates were coated with SE LPS (Sigma Chemical Co.) or ST LPS (Sigma Chemical

Table 3: Breeder box liner and paper pad monitoring for Salmonella

	Treatment Group				
Age (d)	2K	2L2K	3L1K	C	Males
7	+	-	-	-	-
21	+	-	-	-	-
42	+	-	-	-	-
77	-	-	-	-	-
98	-	-	-	-	-
119	-	-	-	-	-

2K = Killed vaccines given on wks 11 and 17; 2L2K = live vaccines given on d 1 and 21 and killed vaccines given on wks 11 and 17; 3L1K = live vaccines on d 1, 21 and wk 11 and 1 killed vaccine given on wk 17. C = non-vaccinated controls; + = positive isolations; - = negative isolations

Co.) at a concentration of 10µg/ml antigens in a carbonate buffer at pH 9.6 were incubated overnight. All plates were coated at the same time from common preparations of SE LPS or ST LPS solutions and controls were run on each sample batch. Based on unpublished pretrial studies, serum samples were diluted at 1:250 and crop and gut lavage samples were diluted at a 1:2 ratio.

Plates were blocked with 0.1*M* PBS plus 0.5ml Tween 20 plus 1% Bovine Serum Albumin (BSA; Southern Biotech, Birmingham, AL) for one h to minimize non-specific binding. Previously diluted samples (serum at a 1:250 ratio and gut or crop lavages at a 1:2 ratio) were added to the microplates along with positive and negative controls and incubated for 90 minutes. Plates were washed two to three times between steps with 0.1M PBS plus 0.5% Tween 20. All incubation steps were done at room temperature and plates placed on mechanical mixer during incubation. Primary antibodies used were mouse anti-chicken IgA (Southern Biotech) diluted 1:1000 or mouse anti-chicken IgG kindly provided by Dr. Peter Holt and diluted 1:40. Primary antibodies were incubated for one h. A secondary goat anti-mouse IgG heavy and light chain specific antibody (Calbiochem, LaJolla, CA) alkaline phosphatase conjugated, at a 1:2000 dilution was added and incubated for one h. Para-nitro-phenyl phosphate chromogen[6] diluted at 1mg/ml in a solution of magnesium chloride and diethanolamine (Sigma Chemical Co.) adjusted to pH 9.8 was added and incubation allowed to proceed for 20-30 minutes under dark conditions. Plates were read at 405nm absorbance with an Ascent (Ascent Lab Systems, Helsinki, Finland) microplate reader.

Statistical analysis: A Log_{10} transformation of OD data was performed and a Completely Randomized Design was used to analyze transformed data, using the General Linear Model procedure of SAS (SAS Institute Inc., Carry, NC) data were analyzed independently within each sampling event (day of breeder age). Means were discriminated using Duncan's multiple range test ($p<0.05$).

Results and Discussion

Salmonella monitoring of chick liners and paper pad results are shown in Table 3. Box liners from female birds were positive for group B *Salmonella* (serovar Heidelberg), indicating hatchery contamination of the females but not the males. The same group B *Salmonella* was isolated from premises housing the 2K group at wk 1, 3 and 6 of age, but no more positive isolates were obtained at wk 11, 14 and 17. Increased age-related resistance as well as increased susceptibility of day-old chicks to *Salmonella* intra-cloacal colonization has been well documented (Cox *et al*., 1990). Low levels of *Salmonella* coming from the hatchery cultured for up to six wks from the environment indicate that these *Salmonella* may have colonized part of the 2K group initially and were probably cleared with time. Although chicks were randomly distributed across pens, no *Salmonella* isolates from the other treatment groups were recovered suggesting that birds in this treatment group were possibly subject to low level exposure to this field isolate. Although this limited natural contamination is a potentially confounding parameter in determining effects of vaccine treatments, data from the study appears to be relatively consistent and since no significant differences were seen between the 2K treatment and control birds through the first 11 weeks, the data should be valid for testing the stated objectives. One observation that must be noted and that may be related to the naturally occurring *Salmonella* in the 2K group is the elevated levels of immunological response after the wk 11 treatment. The elevation in antibody as seen in binding to the ST LPS could be seen as a response to a booster immunization with the natural *Salmonella* serving as the initial exposure.

The focus of the research presented in this paper is on the immunological response in chickens administered live or dead cells of *Salmonella* using common industry practices and procedures for administration of vaccines. It is recognized that different adjuvants, routes of administration and dosages could greatly influence immunological response.

Crop IgA: IgA data are summarized in Fig. 1. Although significant differences were observed between groups during wks 14, 17, 22, 27, 34 and 40, the greatest effect of vaccination treatments on Crop IgA was observed for the 2K group on wk 14. Optical densities for this group were 1.700 and 1.136 on ST and SE LPS plates, respectively. Although no significant differences were observed between the 2L2K and 3L1K groups with respect to controls at 14 wks, ODs were numerically higher for these treatments, with ODs of 0.940 and 0.613 observed for the 2L2K and 3L1K groups.

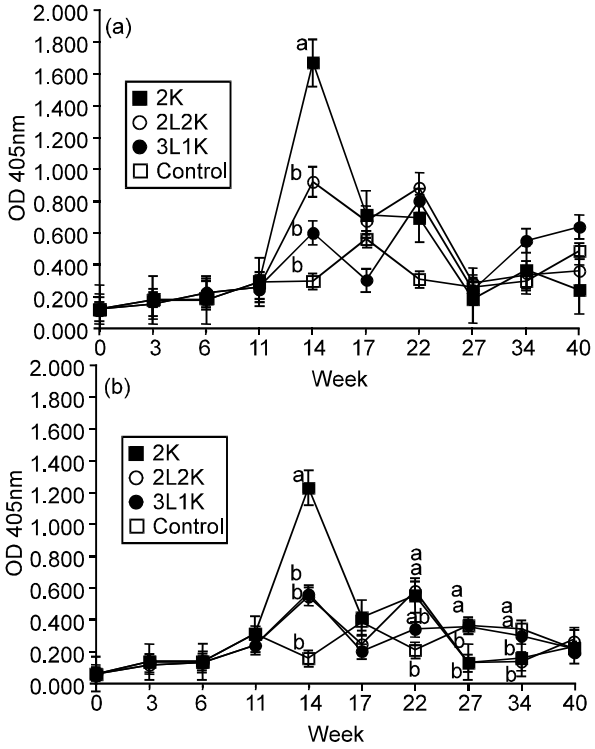

Fig. 1: Optical densities (405nm) of crop IgA assayed on *Salmonella* serovar Typhimurium (a) or *Salmonella* serovar Enteritidis (b) lipopolysaccharide. 2K = Killed vaccines given on wks 11 and 17; 2L2K = live vaccines given on d 1 and 21 and killed vaccines given on wks 11 and 17; 3L1K = live vaccines on d 1, 21 and wk 11 and 1 killed vaccine given on wk 17; C = non-vaccinated controls. N = 10 birds/pen/sample type/sample time. Error bars indicate standard error, p = 0.05

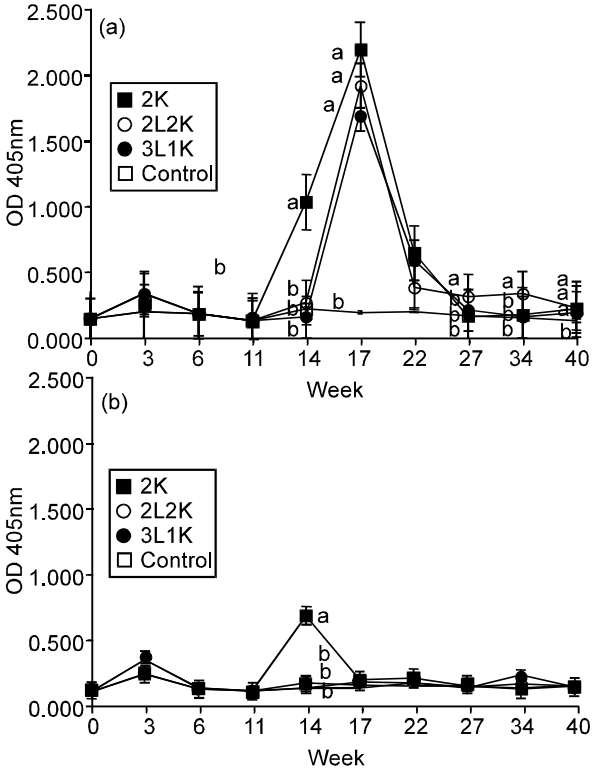

Fig. 2: Optical densities (405nm) of crop IgG assayed on *Salmonella* serovar Typhimurium (a) or *Salmonella* serovar Enteritidis (b) lipopolysaccharide. 2K = Killed vaccines given on wks 11 and 17; 2L2K = live vaccines given on d 1 and 21 and killed vaccines given on wks 11 and 17; 3L1K = live vaccines on d 1, 21 and wk 11 and 1 killed vaccine given on wk 17; C = non-vaccinated controls. N = 10 birds/pen/sample type/sample time. Error bars indicate standard error, p = 0.05

There are no previous reports demonstrating crop IgA responses following one subcutaneous dose of killed antigen, as we observed in this study (samples taken at 14 wks of age). Crop IgA response to oral live antigen exposure has been demonstrated (Seo *et al*., 2002; Seo *et al*., 2003a,b). However, as these investigators noted, no specific antibody-producing cells within the crop have yet been identified and the origin of crop IgA needs to be characterized. Intestinal mucosal IgA peaked at 14-40 d post vaccination when chicks are exposed to orally-administered single dose of killed antigen in microspheres, but not when exposed to these microspheres intramuscularly (Liu *et al*., 2001). Although no positive *Salmonella* isolates were obtained after 6 wks of age, it is also possible that the IgA response obtained may actually be a result of late exposure to low levels of the hatchery isolate, for a longer time than was demonstrable by environmental sampling. This would explain a peak crop IgA response at 14 wks after IM administration of a killed dose 3 wks earlier. If this is the case, our observations would confirm that monitoring crop IgA is a good indicator of recent and low-level exposure to *Salmonella*, as hypothesized earlier (Seo *et al*., 2002). Although every effort was made to avoid bruising and subsequent inadvertent contamination with blood content during crop lavaging and no samples with visible blood in the crop lavage were assayed, high crop IgA may also be due to trace contamination of the lavage sample with blood.

Slightly higher crop IgA at wk 22 (5 wks post second killed vaccination) indicates a weak response to killed vaccine delivery at 17 wks when measured on SELPS, but not when measured on STLPS. Mean ODs on STLPS at this time were higher than mean ODs on SELPS, but lack of significance of 22 wks STLPS crop IgA data was

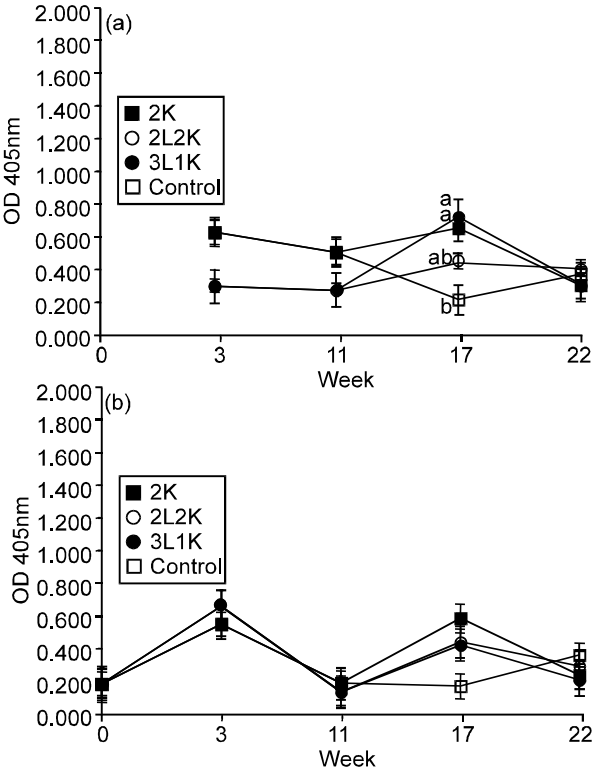

Fig. 3: Optical densities (405nm) of gut IgA assayed on *Salmonella* serovar Typhimurium (a) or *Salmonella* serovar Enteritidis (b) lipopolysaccharide. 2K = Killed vaccines given on wks 11 and 17; 2L2K = live vaccines given on d 1 and 21 and killed vaccines given on wks 11 and 17; 3L1K = live vaccines on d 1, 21 and wk 11 and 1 killed vaccine given on wk 17; C = non-vaccinated controls. N = 10 birds/pen/sample type/sample time. Error bars indicate standard error, p =0.05

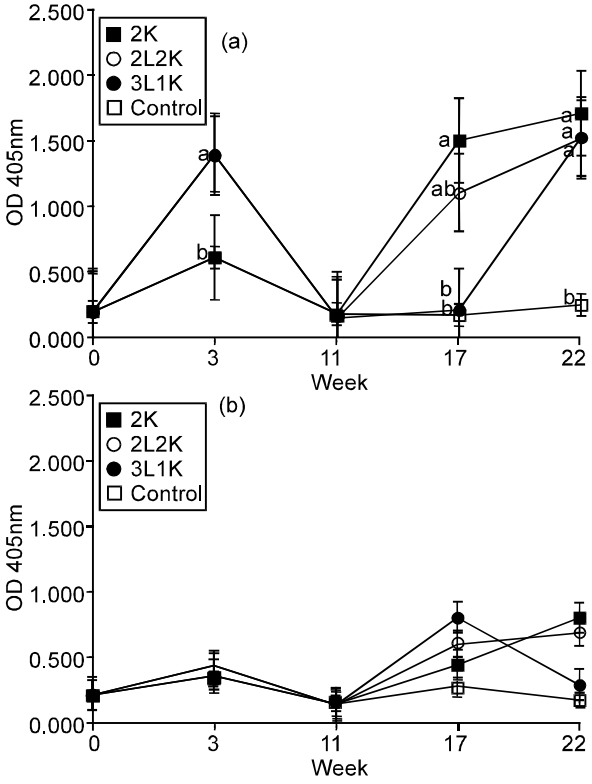

Fig. 4: Optical densities (405nm) of gut IgG assayed on *Salmonella* serovar Typhimurium (a) or *Salmonella* serovar Enteritidis (b) lipopolysaccharide. 2K = Killed vaccines given on wks 11 and 17; 2L2K = live vaccines given on d 1 and 21 and killed vaccines given on wks 11 and 17; 3L1K = live vaccines on d 1, 21 and wk 11 and 1 killed vaccine given on wk 17; C = non-vaccinated controls. N = 10 birds/pen/sample type/sample time. Error bars indicate standard error, p = 0.05

due to higher within group variability. Within group variability was the result of only a fraction of the breeders responding with high crop IgA to the 17 wks killed vaccination. This variability may have to do with differences in the degree of previous exposure to live antigen between treatment birds.

Crop IgG: Crop IgG binding to ST LPS and SE LPS are shown (Fig. 2). For ST LPS, after a first dose of killed antigen at 11 wks, a faster rise in crop IgG of the 2K compared to the 2L2K treatment at wk 14 was observed, but both peaked by 17 wks. The faster rise of crop IgG for the 2K treatment is explained if these birds were previously exposed to field antigen orally, as previously discussed. A second dose of killed vaccine at 17 wks did not elicit a similar crop IgG response (by 22 wks and onward, ODs linger below 0.5). When measured on SE LPS (Fig. 1b), Crop IgG reached a short-lived peak at 14 wks, with no other mean OD's being over 0.500 after 17 wks. These findings seem to indicate that crop IgA and IgG are short lived in time when compared with serum IgA and IgG levels and that oral exposure to antigen is a requirement for raising the crop concentration of both antibody isotypes. The differences in OD's between SE LPS and ST LPS assays would indicate that responses were primed by a (live) *Salmonella* serovar Typhimurium (which is the live vaccine strain), or a closely related (the hatchery-associated group B) serovar.

Gut IgA: Gut IgA binding to SE LPS was measured only at day-of-age (wk 0), due to the small amount of lavage sample available (Fig. 3). No differences among treatments throughout time were noted on gut IgA binding to SE LPS, although OD's were slightly higher for all treatments on wks 3 and 17. On these wks, ODs of all vaccinated groups were numerically higher than

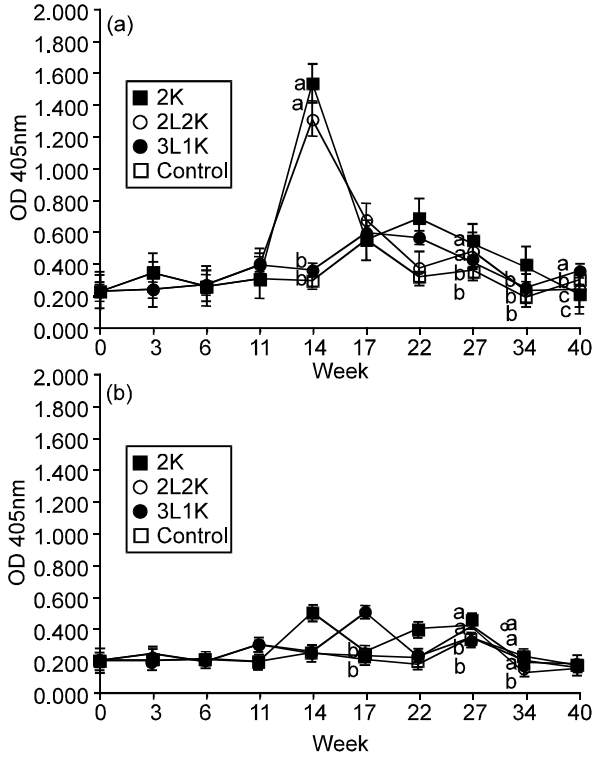

Fig. 5: Optical densities (405nm) of serum IgA assayed on *Salmonella* serovar Typhimurium (a) or *Salmonella* serovar Enteritidis (b) lipopolysaccharide. 2K = Killed vaccines given on wks 11 and 17; 2L2K = live vaccines given on d 1 and 21 and killed vaccines given on wks 11 and 17; 3L1K = live vaccines on d 1, 21 and wk 11 and 1 killed vaccine given on wk 17; C = non-vaccinated controls. N = 10 birds/pen/sample type/sample time. Error bars indicate standard error, p = 0.05

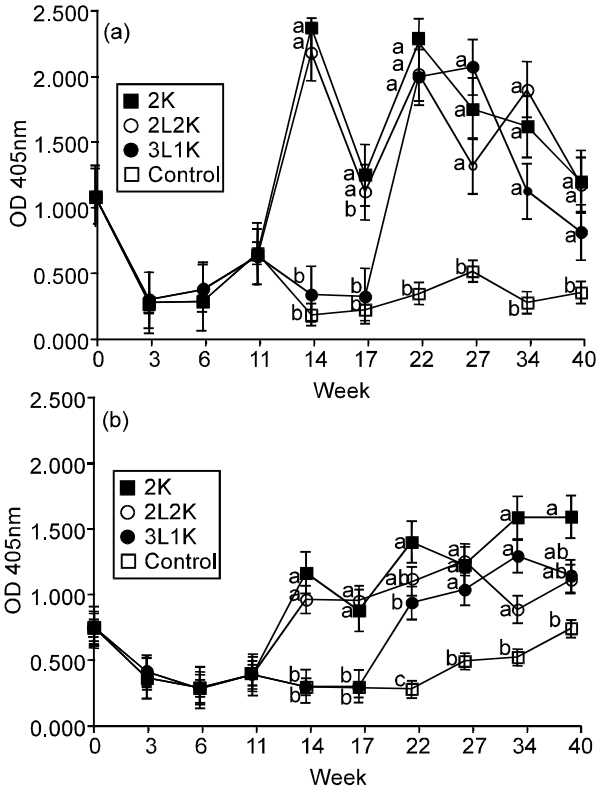

Fig. 6: Optical densities (405nm) of serum IgG assayed on *Salmonella* serovar Typhimurium (a) or *Salmonella* serovar Enteritidis (b) lipopolysaccharide. 2K = Killed vaccines given on wks 11 and 17; 2L2K = live vaccines given on d 1 and 21 and killed vaccines given on wks 11 and 17; 3L1K = live vaccines on d 1, 21 and wk 11 and 1 killed vaccine given on wk 17; C = non-vaccinated controls. N = 10 birds/pen/sample type/sample time. Error bars indicate standard error, p = 0.05

the controls. Higher ODs for gut IgA would be expected following exposure to oral (live) antigen. When binding was to ST LPS, gut IgA ODs for the 3L1K and 2K treatments were higher on wk 17 compared to the control. The 2L2K treatment had a numerically higher OD than the control, but was not statistically different from either the control or the other vaccinated groups.

Gut IgG: Differences among treatments were noted only on measurements of binding to ST LPS (Fig. 4). Peak ODs were observed on wks 3, 17 and 22. Chicks receiving the live vaccine had higher gut IgG by wk 3, but these concentrations dropped to control levels by wk 11, regardless of a second administration of live vaccine at wk 6. A killed vaccine given at wk 11 was capable of raising gut IgG levels by wk 17, regardless of previous live priming, as seen by higher gut IgG for the 2K and 2L2K treatments. In contrast, birds receiving only live vaccines were not able to sustain a high gut IgG response by wk 17, as seen by the low 3L1K OD. By wk 22, all vaccinated groups had received at least 1 killed vaccine by wk 17 and consequently showed higher gut IgGs. These findings indicate that gut oral live vaccine elicits a short-lived gut IgG response.

Serum IgA: Although differences for serum IgA were obtained on wks 14, 27, 34 and 40 (Fig. 5), the numerically highest was obtained by wk 14, for the 2K and 2L2K groups receiving a killed vaccine previously (wk 11). This peak was detected 3 wks post vaccination, only when binding was to ST LPS and no comparable peak was observed thereafter. No peaking ODs were observed when sampling at wk 22, 5 wks after the second killed vaccine (wk17). Although other investigators have reported serum IgA peaking up to 6

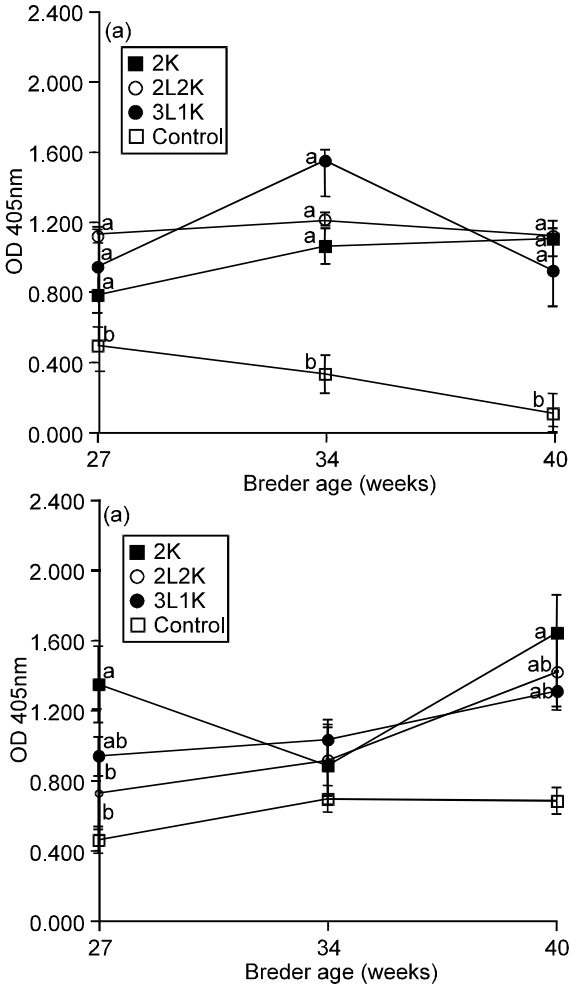

Fig. 7: Optical densities (405nm) of yolk IgG assayed on *Salmonella* serovar Typhimurium (a) or *Salmonella* serovar Enteritidis (b) lipopolysaccharide. 2K = Killed vaccines given on wks 11 and 17; 2L2K = live vaccines given on d 1 and 21 and killed vaccines given on wks 11 and 17; 3L1K = live vaccines on d 1, 21 and wk 11 and 1 killed vaccine given on wk 17; C = non-vaccinated controls. N = 10 birds/pen/sample type/sample time. Error bars indicate standard error, p = 0.05

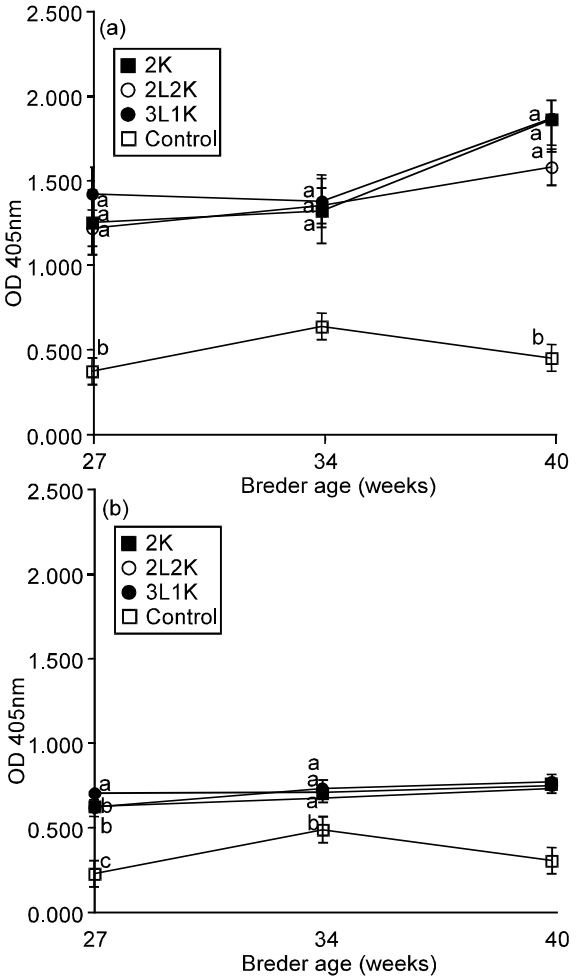

Fig. 8: Optical densities (405nm) of hatchling serum IgG assayed on *Salmonella* serovar Typhimurium (a) or *Salmonella* serovar Enteritidis (b) lipopolysaccharide. 2K = Killed vaccines given on wks 11 and 17; 2L2K = live vaccines given on d 1 and 21 and killed vaccines given on wks 11 and 17; 3L1K = live vaccines on d 1, 21 and wk 11 and 1 killed vaccine given on wk 17; C = non-vaccinated controls. N = 10 birds/pen/sample type/sample time. Error bars indicate standard error, p = 0.05

wks post vaccination (Liu *et al*., 2001), we were unable to show a comparable long-lasting serum IgA response. All vaccinated treatments showed slightly higher ODs when compared to controls at varying times throughout wks 27 to 40, but none were consistently higher and numerical differences though statistically significant, were relatively small in magnitude.

Serum IgG: Optical densities for serum IgG are reported in Fig. 6. Initial high titers of serum IgG at day of age were detected and were maternally derived, since the 57 wk-old female parent stock had been vaccinated twice with an autogenous bacterin, containing groups B and D1 Salmonellae. These titers waned as expected by 3 wks of age. Killed but not live vaccination elicited the highest serum IgG responses as seen on wk 14 for the 2K and 2L2K groups and wks 22 and after, for all vaccinated groups. Response to only one killed vaccine diminished faster than for 2 killed vaccines, as seen by the decline in ODs by wk 17 of 2K and 2L2K treatments and by a numerically (not statistically) faster decline of

the 3L1K group by wks 22, 34 and 40. Although all treatments were different from controls throughout wk 40, the rate of decline in ODs seems to suggest that IgG titers would not last throughout a normal 65-wk production period.

Yolk and hatchling serum antibodies: Only yolk IgG (Fig. 7) but no yolk IgA was detected (data not shown). These results were expected, since IgG is deposited in the hen's maturing follicle, whereas IgA is deposited in the amniotic fluid. Egg yolk IgG was higher for all vaccinated groups throughout all wks sampled. Hatchling Serum IgA (data not shown) and IgG (Fig. 8) followed egg yolk trends, with no detectable IgA and higher IgG for hatchlings from vaccinated treatments throughout all sampling periods. IgG levels in yolk and hatchling sera were maintained through time.

Finally, ELISA responses are clearly dependent on the antigen type used, as can be seen in general differences in profiles when using ST or SE LPS. When adapting a particular ELISA procedure for field monitoring, it would be best to choose an LPS group-compatible with the most common serovar encountered in the field.

References

Arnold, J.W. and P.S. Holt, 1995. Response to *Salmonella-enteritidis* infection by the immunocompromised avian host. Poult. Sci., 94: 656-665.

Cason, J.A., N.A. Cox and J.S. Bailey, 1994. Transmission of *Salmonella* Typhimurium during hatching of broiler chicks. Avian Dis., 38: 583-588.

Chatfield, S.N., N. Fairweather, I. Charles, D. Pickard, M. Levine, D. Hone, M. Posada, R.A. Strugnell and G. Dougan, 1992. Construction of a genetically defined *Salmonella typhi* Ty2 *aroA*, *aroC* mutant for the engineering of a candidate oral typhoid-tetanus vaccine. Vaccine, 10: 53-60.

Cox, N.A., J.S. Bailey and L.C. Blankenship, 1990. Fifty percent colonization dose for *Salmonella* Typhimurium administered orally and intracloacally to young broiler chicks. Poult. Sci., 69: 1809-1812.

Curtiss III, R., R.M. Goldschmidt, N.B. Fletchall and S.M. Kelly, 1988. Avirulent *Salmonella typhimurium) cya) crp* oral vaccine strains expressing a streptococcal colonization and virulence antigen. Vaccine, 6: 155-160.

Dougan, G.S., D. Maskell, D. Pickard and C.E. Hormaeche, 1987. Isolation of stable *aroA* mutants of *Salmonella typhi* Ty2: Properties and preliminary characterization in mice. Mol. Gen. Genet., 207: 402-407.

Dougan, G.S., D. Chatfield, J. Pickard, D. Bester, D. O'Callaghan and D. Maskell, 1988. Construction and characterization of vaccine strains of *Salmonella* harbouring mutations in two different *aro* genes. J. Infect. Dis., 158: 1329-1335.

Hassan, J.O. and R. Curtiss III, 1994. Development and evaluation of an experimental vaccination program using a live avirulent *S. typhimurium* strain to protect chickens against challenge with homologous and heterologous *Salmonella* serotypes. Inf. Imm., 62: 5519-5527.

Hassan, J.O. and R. Curtiss III, 1997. Efficacy of a live avirulent *Salmonella typhimurium* vaccine in preventing colonization and invasion of laying hens by *Salmonella typhimurium* and *Salmonella enteritidis*. Poult. Sci., 62: 30-37.

Holt, P.S. and R.E. Porter Jr., 1993. Effect of induced molting on the recurrence of a previous *Salmonella enteritidis* infection. Poult. Sci., 72: 2069-2078.

Holt, P.S., L.E. Vaughn, R.K. Gast and H. Stone, 2002. Development of a lavage procedure to collect crop secretions from live chickens for studying crop immunity. Avian Path., 31: 589-592.

Hone, D.M., A.M. Harris, S. Chatfield, G. Dougan and M.M. Levine, 1991. Construction of genetically defined double *aro* mutants of *Salmonella typhi*. Vaccine, 9: 810-816.

Hosieth, S.K. and B.A.D. Stocker, 1981. Aromatic-dependant *Salmonella typhimurium* are non-virulent and effective as live vaccines. Nature, 291: 238-239.

Idexx, 2004. Flockchek® *Salmonella enteritidis* antibody test kit. Page 16 in Poultry and Livestock Catalog and ELISA technical guide. IDEXX Laboratories, Inc. Westbrook, Maine.

Kaiser, M.G. and S.J. Lamont, 2001. Genetic line differences in survival and pathogen load in young layer chicks after *Salmonella enterica* serovar *enteritidis* exposure. Poult. Sci., 80: 1105-1108.

Kaiser, M.G., N. Deeb and S.J. Lamont, 2002. Microsatellite markers linked to *Salmonella enterica* serovar *enteritidis* vaccine response in young F-1 broiler-cross chicks. Poult. Sci., 81: 193-201.

Lee, G.M., G.D.F. Jackson and G.N. Cooper, 1983. Infection and immune responses in chickens exposed to *Salmonella typhimurium.* Avian Dis., 27: 577-583.

Lillehoj, H.S. and M. Okamura, 2003. Host immunity and vaccine development to coccidia and *Salmonella* infections in chickens. J. Poult. Sci., 40: 151-193.

Liu, W., Y. Yang, N. Chung and J. Kwang, 2001. Induction of humoral immune response and protective immujnity in chickens against *Salmonella enteritidis* after a single dose of killed bacterium-loaded microspheres. Avian Dis., 45: 797-806.

McCapes, R.H. and H.P. Riemann, 1998. Obstacles to Control of *Salmonella* in Foods of Avian Origin. Pages 23-42 in: Proceedings of the International Symposium on Food-Borne *Salmonella* in Poultry. American Association of Avian Pathologists, Baltimore, Maryland.

Richardson, L.J., B.W. Mitchell, J.L. Wilson and C.L. Hofacre, 2003a. Effect of an electrostatic space charge system on airborne dust and subsequent potential transmission of microorganisms to broiler breeder pullets by airborne dust. Avian Dis., 47: 128-133.

Richardson, L.J., C.L. Hofacre, B.W. Mitchell and J.L. Wilson, 2003b. Effect of electrostatic space charge on reduction of airborne transmission of *Salmonella* and other bacteria in broiler breeders in production and their progeny. Avian Dis., 47: 1352-1361.

Schat, K.A. and T.J. Myers, 1991. Avian intestinal immunity. Crit. Rev. Poult. Biol., 3: 19-34.

Seo, K.H., P.S. Holt, R.E. Brackett, R.K. Gast and H.D. Stone, 2002. Mucosal humoral immunity to experimental *Salmonella* enteritidis infection in the chicken crop. Avian Dis., 46: 1015-1020.

Seo, K.H., P.S. Holt, L.E. Vaughn, R.K. Gast and H.D. Stone, 2003a. Detection of *Salmonella enteritidis*-Specific Immunoglobulin A antibodies in crop samples from chickens infected with *Salmonella enteritidis*. Poult. Sci., 82: 67-70.

Seo, K.H., P.S. Holt, R.K. Gast and H.D. Stone, 2003b. Simple and rapid methods for detecting *Salmonella enteritidis* in raw eggs. Int. J. Food Micro., 87: 139-144.

United States Department of Agriculture, Food Safety Inspection Service, 1996. Pathogen Reduction; Hazard Analysis and Critical Control Point (HACCP) Systems; Final Rule. Federal Register, 144: 38306-38989.

Zhang-Barber, L., A.K. Turner and P.A. Barrow, 1999. Vaccination for control of *Salmonella* in poultry. Vaccine, 17: 2538-2545.

Abbreviation Key: 2K = two killed vaccines; 2L2K = two live and two killed vaccines; 3L1K = three live and 1 killed vaccine; CL = crop lavage; GL = gut lavage; OD = optical density; SELPS = *Salmonella enteritidis* lipopolysaccharide; SER = serum; ST = *Salmonella typhimurium*; STLPS = *Salmonella typhimurium* lipopolysaccharide